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THEORETICAL MODELS OF CYST FORMATION AND GROWTH

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Abstract

Although the formation of fluid-filled, epithelial-lined cysts is a common event in a variety of tissues, the mechanisms involved are not well understood. Discussed here are means by which those mechanisms might be elucidated.

In general, there are too few data available for complete analysis of in vivo disease processes. It can be suggested only that epithelial proliferation and basement membrane growth are probably absolute requirements. Whether the forces for fluid accumulation precede or follow the stimuli for cell growth cannot be stated with certainty. On the other hand, in certain in vitro model systems the forces required to keep cyst cavities filled with fluid may be so small that cell growth, rather than fluid accumulation, seems the more likely primary event.

Key Words: Renal cysts, epithelial cysts, fluid accumulation, proliferation.

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Introduction

Cysts are known to occur in a wide variety of tissues and often in several tissues simultaneously. They occur as normal phenomena or in the context of naturally occurring or experimentally induced disease states. Some of the cystic diseases are congenital or hereditary, both dominant and recessive, while others are acquired. Cystic change recently has been observed even in cell cultures and thus is considered to be a fairly common phenomenon. Although discussions of this topic date even from the 19th century and several of the more obvious and basic facts are now well established, there is still no clear choice among the several hypotheses proposed to explain the occurrence of cysts. That is, the mechanisms involved in the initial formation and in the subsequent growth and development of cystic spaces remain poorly understood. The purpose here is to expand upon the known facts and to arrive at some plausible possibilities for proceeding further toward a solution to the problem. This paper will be concerned mostly with cysts which arise in kidney tubules. It is likely, however, that the principles have wider applicability.

Basic Mechanisms

The cysts of interest here are always filled with fluid and are always limited by an epithelial layer resting on a basement membrane. This fact brings us immediately to a most basic question, namely, does the abnormality lending to cyst formation lie principally in the growth properties of the limiting membrane or in the forces lending to fluid accumulation? That is, does epithelial growth start first and, by producing cells with the capacity for net fluid secretion or simply by forcing an increase in the surface area of a spherical shell, somehow generate forces sufficient to keep that shell filled with ambient fluid or, alternatively, does the accumulation of fluid in a performed tissue space somehow stretch or otherwise induce the limiting epithelial layer to grow in surface area just sufficient to keep up with increasing cyst volume? In theory at least, the cell growth-first possibility should be easily separable from the fluid-first possibility on the basis of fairly simple observations.

Cell Proliferation Model

Consider first the possibility that a previously stable epithelial layer is induced to grow further, or that a normally developing epithelial layer simple

fails to stop growing when normal size has been reached. The latter might be the case, for example, in a congenital cystic disease. We will assume that the cells maintain a fairly constant size and that the proliferating epithelium remains predominantly as a single layer structure which forms neither surface folds or polyps of significant size nor multilayered tumor masses. The pattern of growth of an ideal spherical cyst now can be predicted to be exponential. This comes about because the surface area of the cyst would be proportional to the number of cells present at any given time. Therefore, if cell division were a primary event, the time rate of change of the number of cells and the time rate of change of cyst wall surface area would be proportional to the number and area at that time. The resulting curve thus would be exponential of the form $A = A_o \exp(kt)$ where A_o is the initial area and k is the rate constant. That constant is related to the time t required for the number of cells to double and in turn might be influenced by environmental circumstances such as resistance to cyst enlargement afforded by the surrounding matrix or interstitial tissue. Nonetheless, if cyst growth is a result of such cell multiplication, it still follows that the radius, surface area, and volume of the cyst would be exponential functions of time whose rate constants would be k/2, k, and 3k/2, respectively. The case thus would have unique characteristics and to confirm the possibility for an in vivo situation one would need only to observe a cyst for some period of time and to find that cyst radius, surface area, and volume all vary with time in the required exponential manner.

Fluid Accumulation Model

Consider now the alternative, second possibility in which fluid accumulation is considered to be the primary event in cyst formation, with cell growth following only as a consequence. The cyst growth patterns again are predictable in theory. Namely, if fluid accumulation were driven by osmotic and/or hydrostatic forces and if those forces remained constant with time, the radius of the cyst would increase as a linear function of time while the surface area and the volume would be quadratic and cubic functions of time, respectively. If the initial osmotic force were conceived to decay with time (e.g., by dilution of intra-cystic osmotic solutes) or even to increase with time (e.g., by continuing enzymatic breakdown of trapped macromolecules), the growth curves would become asymptotic or non-linear. Similar effects might result from changes in the deformability of the para-cystic matrix. It is unlikely, however, that a combination of driving forces for or resistance to fluid accumulation could exist or persist for sufficient time to generate an exponential growth curve for a population of cysts. Again, these possibilities might be investigated by simple in vivo observations.

Having now developed the means for dissecting the possible causes of cyst formation, the next logical step normally would be to examine the reported observations on cyst growth characteristics and to reach some logical conclusions. Unfortunately, however, for the case of naturally occurring and experimental renal cystic disease in vivo, we have little data upon which to proceed. Perhaps surprisingly, the data on cyst growth rate simply are not available. Nonetheless, it is of some value to consider what is known about renal cystic disease and in that way at least examine and possibly exclude certain possible mechanisms.

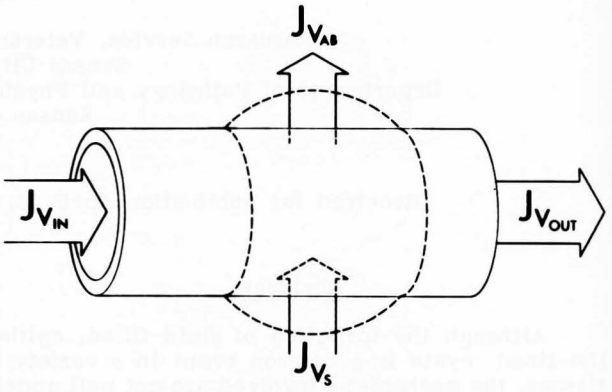

Figure 1. Hypothetical nephron segment pre-disposed to cystic dilation. Arrows represent volume flows (see text). (Reproduced from ref. 16, with permission).

Renal Cysts

The hypothetical conditions under which renal cysts develop in vivo are summarized in Figure 1. Shown there is a nephron segment whose central region is predisposed to cyst formation by some as yet unknown mechanism. The several arrows represent volume flows. JV_{in} is the proximal inflow and, for a given kidney tubule, is equal to the single nephron glomerular filtration rate plus or minus any secretory or reabsorptive flux that may have occurred upstream. Mostly because of upstream reabsorption, reasonable values for JV_{in} range from about 5 to about 50 nl/min. At the opposite end of the tubule, JV_{out} is simply the distal outflow. It will range from zero during complete outlet obstruction to values greater than or less the JV_{in} depending upon the possibilities of intervening fluid absorption out of the tubule lumen, JV_{ab}, or fluid secretion into the tubule lumen, JV_s. Typical normal values for JV_{out} would range from about 2 to about 20 nl/min. In any case, the net effect is summarized by the equation $(JV_{in} - JV_{out} + JV_s - JV_{ab}) = dV/dt$ in which the change in cyst volume V with time t is simply the algebraic sum of the several independent volume flows.

Balloon Model

Historically, the most logical explanation for cyst formation in renal tubules was thought to be distal tubule obstruction in the face of continuing glomerular filtration. Presumably, the tubules would then dilate like so many balloons. Let us examine, therefore, what would happen if a tubule in fact were totally obstructed distally and if the wall of the developing cyst were impermeable and inert. Clearly, this is a fluid-first type of situation and, if the glomerular filtration rate were truly constant with time, the cyst volume would grow linearly with time. Then, if JV_{in} were of the order of 5 nl/min, as seems reasonable for distal tubule segments, spherical cysts of volumes 0.1, 1, 10, and 100 ml

could be produced in 14 days, 140 days, 3.8 years, and 38 years, respectively. If JV_{in} were of the order of 50 nl/min, as seems reasonable for proximal tubule segments, cysts of the various sizes would be produced 10-times faster. As it turns out, all of these times are reasonable, both in some human renal cystic diseases and in some experimental cystic diseases in animals [3, 6, 9, 16]. Before these calculations are taken as evidence favoring the fluid-first theory of cyst formation, however, there are several other facts that must be considered.

First, when a balloon is stretched its surface area increases at the expense of wall thickness. Thus, if cell and basement membrane mass were to remain constant during cyst formation, the original cell height of 7.5 μm and the original basement membrane thickness of 0.25 μm in an average renal tubule would become attenuated to 0.25 and 0.01 μm, respectively, in cysts only 1 mm in diameter and would be attenuated to angstrom dimensions in cysts just 1 cm in diameter. In fact, however, numerous observations [3, 5, 6, 10] have shown the cyst wall epithelial cells not to be thinner than normal but rather to be similar in size to the normal lining cells of nephrons. Furthermore, the tubule basement membrane is found more often to be normal or even increased in thickness than to be thinned.

Second, the normal renal tubule has a limited stretchability, mostly because of its basement membrane [15]. This is true and the tubule distensibility is similar both for isolated tubules in vitro [15] and for tubules surrounded by normal renal tissue in vivo [4, 13]. The result is that a change in outer tubule diameter of nearly 40%, from about 35 to about 50 μm, can be accomplished by changes in transtubular pressure within a physiological range of about 5 to 40 cm H_2O. However, the diameter-pressure curve flattens off near the latter pressure and significant further tubule enlargement would require unphysiologic pressures far larger than the few cm H_2O luminal pressures sometimes but not always observed in the usual context of cyst formation. Furthermore, even if the basement membrane were assumed, as previously suggested [3], to be defective and abnormally stretchable, one still would be constrained by the problem of unacceptable cellular and basement membrane thinning. In any case, recent studies indicate that basement membrane stretchability probably is not abnormal in the walls of renal cysts [11].

Hyperplasia Model

For both of the reasons just mentioned, i.e., the lack of observed cellular or basement membrane thinning to the predicted degree and the improbable role of an abnormal, more easily stretchable basement membrane, it becomes apparent that cellular proliferation and basement membrane production are important aspects of cyst formation in the kidney. However, whether this knowledge helps in choosing between the fluid-first hypothesis and the cell growth-first hypothesis of cyst formation remains to be seen.

There is ample evidence that cellular growth occurs in the context of cyst formation. In addition to the logical arguments just stated, there are several reports of the formation of small epithelial polyps on the walls of cysts [6, 7, 10, 12]. Such polyps clearly show the propensity of the epithelium for proliferation, at least focally. At the same time, however, these polyps have been observed at the distal ends of fusiform cystic spaces and in such positions that they might cause outlet obstruction. Again, therefore, it is not clear which event is more important to the eventual formation of the cyst, the proliferation itself, as in the cell growth-first hypothesis, or the obstruction with fluid accumulation, as in the fluid-first hypothesis. Perhaps the two events could even be coordinated rather than independent.

As an example of the possible interplay between obstruction and cellular hyperplasia, consider the events depicted in Figure 2. At left is the normal situation with about 8 cells around the circumference of a tubule at a transmural pressure of 10 cm H_2O. In the middle panel a distal obstruction has caused a moderate increase in the tubule diameter that is well within the range allowed by normal basement membrane stretchability. Note, however, that the cells are both flattened and stretched, a situation known from cell culture work to predispose to cellular proliferation [2, 8]. The right panel shows the effect of such proliferation, there now being 9 rather than 8 cells per tubule circumference. Note now that each of the 9 cells has been able to revert to approximately normal dimensions, thus both reducing the presumed stimulus for proliferation and, by increasing the cellular and basement membrane mass, decreasing the basement membrane stretch and allowing the transmural pressure to return to normal.

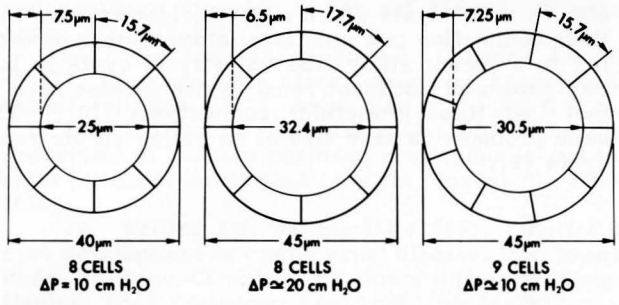

Figure 2. Suggested manner of tubule cell growth induced by tubule dilation (Reproduced from ref. 16, with permission).

Absorption, Secretion, and "Saturation"

It would seem in the preceding example that if the distal obstruction were constant, the repeated cycle of tubule pressure increase, cell stretching, cell division, and return toward normal pressure might continue indefinitely and with the result of continuing tubule enlargement in the absence of unacceptable degrees of wall attenuation or hydrostatic pressure. The basic stimulus for these cycles would be fluid accumulation, clearly a fluid-first situation. However, as will be explained next, it is unlikely that such cycles could continue for long without a fundamental change in cellular properties not yet mentioned to this point in our discussion. Those properties have to do with the intrinsic ability of the cell to transport fluid, either absorption or secretion.

The scenarios considered thus far assumed that the fluid flow into the cystic space is constant, as if by a constant glomerular filtration, and that the outflow is zero, as if by total distal obstruction of an

inert tubule segment. However, the cells that lined the originally normal tubule segment most typically would have had a considerable capacity for fluid absorption. Thus, if that absorptive capacity were maintained as the epithelium proliferated to cover the ever enlarging inner surface area of the cyst cavity, a point would be reached at which all inflow into the cyst cavity would be reabsorbed across the cyst wall and no further growth of the cyst size could occur. This case, which we have termed "saturation" [16], is shown by the dashed line in Figure 3. Sample calculations suggest that if cellular transport properties were "normal" on a per cell basis in cysts of proximal tubule origin, those cysts would level off or "saturate" at volumes of about 0.2 ml and diameters somewhat less than 1 mm. However, those volumes and diameters are quite small relative to common experience with human and experimental disease. On the other hand, for the cysts to be larger than "saturation", one would have to postulate reduced epithelial absorptive capacity or even secretion. For example, if epithelial transport were zero and JV_{in} in the form of glomerular filtration were constant, the solid line in Figure 3, linear growth curves would be followed with seemingly no limit except time on the eventual size of the cyst. Alternatively, or in addition to a fixed glomerular filtration, the epithelial cells might actually secrete fluid into the cyst cavity, a case shown in Figure 3 by the dotted line. Secretion must, of course, be the case in non-renal cysts with no equivalent to glomerular filtration and also in renal cysts which might incidently have lost their connection to a functional glomerulus. According to a recent study, the majority of cysts in human autosomal dominant renal cystic disease have in fact lost those glomerular connections [10]. The same probably is true also of so-called simple renal cysts [1].

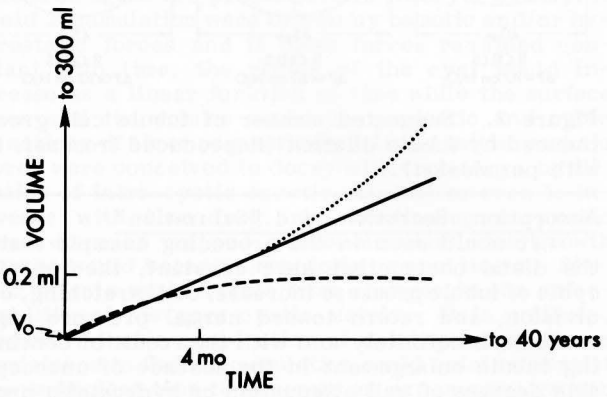

Figure 3. Possible cyst growth patterns. Times and volumes are arbitrary (Reproduced from ref. 16, with permission).

Cysts in Cell Culture

Finally, it is of value to consider briefly the recently described model system in which MDCK cells cultured in 3-dimensional gel matrix form clonal growths in the form of enlarging spherical shells [14]. A growth curve for one such culture is shown in Figure 4. Three important observations can be made. First, the growth curve has at least two components indicating an initial growth process and a subsequent "attenuation" process during which growth rate slows and apparently stops. Second, the initial growth rate fits a single exponential curve and, if the surface area of the cysts is proportional to the number of cells, the corresponding exponential increase fits to a cell doubling time of 2 to 2.5 days, Figure 5. This would be consistent with direct observations of the rate of initial cell proliferation in such cultures and clearly is consistent with a cell growth-first hypothesis for cyst development. As also shown in Figure 5, other studies suggest that this doubling time might also be a function of different culture conditions, one of which might be the resistance to cyst enlargement afforded by the gel matrix. The third observation is that when one estimates, from the growth curve, the maximum rate of fluid entry across an epithelial area, and then assigns to the epithelium a hydraulic conductivity of the magnitude observed for renal tubule epithelia in vivo, the driving force necessary for filling the culture cysts is found probably to be very small, of the order of 0.2 cm H_2O hydrostatic pressure gradient or 0.01 mOsm osmotic solute gradient. It seems, therefore, that the ability of fluid to enter the cyst cavity could hardly be a limiting factor and, conversely, that the fluid-first hypothesis is unlikely to apply in this experimental situations. The source of fluid here, of course, would presumably be secretion by the ever-enlarging area of cyst epithelium although, again, the required forces may be so small that little more than simple diffusion would be required.

Conclusions

In conclusion, in exploring some logical means for evaluating the mechanisms of cyst growth it is found that, in general, too few data are available to reach firm conclusions for actual experimental or natural models of cystic disease. Nonetheless, from circumstantial evidence it can be concluded that cell growth and basement membrane growth are probably absolute requirements. In an experimental, in vitro model it also is likely that the forces required to keep the cyst cavity filled with fluid are very small and that cell growth rather than fluid accumulation is the more likely primary event. Beyond such simple statements, however, one can only speculate and hope for the future accumulation of appropriate data. Such data would include at least the average sizes and the distribution of sizes of cysts in actual cystic disease samples collected at numerous time intervals during the course of a cystic disease process. In model systems, both in vivo and in vitro, one would also hope for new data regarding cellular proliferative ability and quantitative measurements of cell sizes and numbers per unit area of cyst surface. The question of the functional capacity of cyst lining cells must also be addressed, both the absorptive capacity as would be important in the context of "saturation", and the secretory capacity as would be pertinent for model or tissue systems without obvious (e.g., glomerular filtration) or reliable sources for cyst fluid. Finally, since some measure of cell proliferation seems clearly to be involved in cyst formation, much of the future may lie in elucidating the stimuli for and the kinetics of cell multiplication under conditions of cyst formation.

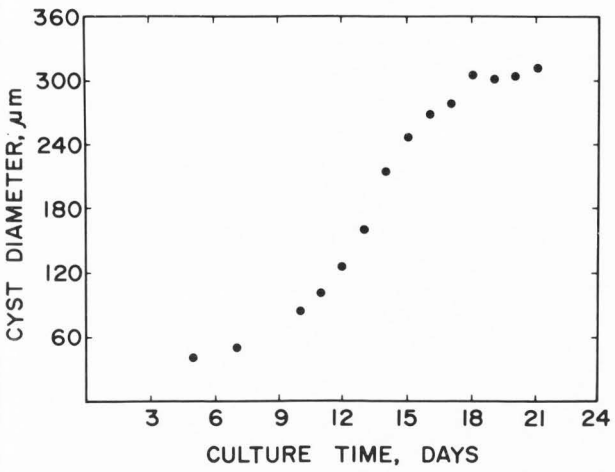

Figure 4. Sample growth curve for MDCK cysts in gel matrix.

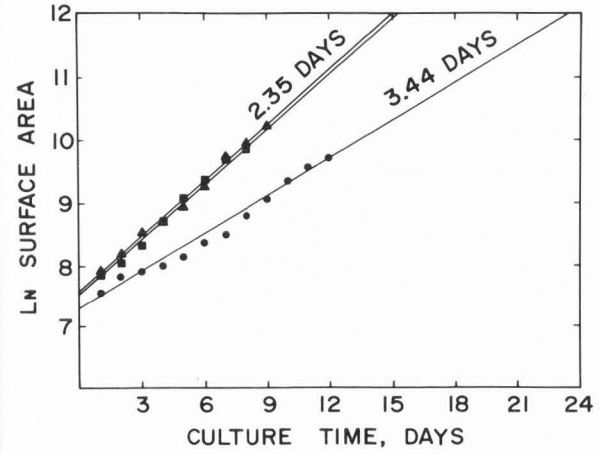

Figure 5. Relationship between outer surface area and culture time for MDCK cysts in gel matrix. Cell doubling times of 2.35 and 3.44 days are calculated from the slopes of these data for three different cultures.

Acknowledgments

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Discussion With Reviewers

G. E. Striker: Previous publications from this group and others emphasized the fact that at least some of these cysts "function," i.e., they secrete and reabsorb. These observations seem to be downplayed in this review. Are there additional unpublished observations which the authors have which are relevant to this point?

Authors: Reports of function are based on the composition of the cyst fluid. That is probably inadequate for the present purposes and we therefore suggest that future, more detailed studies be performed.

K. Gardner: It seems likely that interstitium, enlarging adjacent cysts, and the renal capsule offer resistance to the growth of any single cyst in vivo. Under such circumstances why does epithelial proliferation occur circumferentially rather than intralumenally, the direction in which resistance to proliferation would intuitively seem to be lower?

Authors: We don't know what the forces are that cause papillary ingrowth. They may or may not have

anything to do with ambient physical conditions, e.g. cystic, solid, and papillary tumors from the some cell line may all recur in the same area. The force for papillary growth direction might even be greater than that for 2 - dimensional growth. It seems that most epithelia like to stay 2 - dimensional

K. Gardner: Elevated intracystic hydrostatic pressures have been measured in vivo in both human and experimental renal cystic disease (cf. Refs 2, 4 & 5). How can proliferation, as the primary event in cyst growth, lead to elevated intracystic pressures? Are not these observations more compatible with the thesis that fluid accumulation is primary and that proliferation occurs as a consequence of cyst-wall stretching?

Authors: If cell growth were the only thing that happened during cyst development, one might expect the luminal pressure to even be negative (relative to interstitium). However, if the new cells were even a little bit secretory, the cyst pressure could easily be positive. The same is true if there is some osmotic force into the lumen. Positive luminal pressure there does not exclude proliferation as the premary event.